Bioefficacy of Enzyme Preparations Containing β-Glucanase and Xylanase Activities in Broiler Diets Based on Barley or Wheat, in Combination with Flavomycin[1]

E. ESTEVE-GARCIA,* J. BRUFAU,* A. PÉREZ-VENDRELL,* A. MIQUEL,* and K. DUVEN†

*Department of Animal Nutrition, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Centre de Mas Bové, Apartat 415, 43280 Reus, Spain, and †Hoechst Roussel Vet GmbH, Rheingaustrasse 190, D-65203, Wiesbaden, Germany

ABSTRACT The objective of the study was to determine the effects of two enzyme preparations containing β-glucanase and xylanase activities on barley- and wheat-based diets, respectively, for broilers, in combination with flavomycin. In addition, the stability of the enzyme preparations after pelleting was measured. Temperatures recorded during the pelleting process reached 75 to 80 C, and the activities recovered with respect to the amounts present in the mash feed before pelleting were 80% or higher.

Two performance experiments were conducted simultaneously under the same conditions over 6 wk. In addition, intestinal viscosity and incidence of vent pasting were measured and carcasses were eviscerated to determine abdominal fat, carcass yield, and percentage weight of intestines and viscera. Twenty-four pens (12 per sex), each containing 75 chickens were used in each experiment. Wheat- or barley-based diets were supplemented with flavomycin and a xylanase or a β-glucanase preparation, respectively, in a 2 × 2 factorial arrangement of treatments. In the wheat diets, xylanase and flavomycin improved feed efficiency, in parallel with a reduction of intestinal viscosity. Xylanase reduced the incidence of vent pasting and the percentage viscera, especially of intestines, and increased abdominal fat. In the barley diets, β-glucanase and flavomycin improved feed conversion. β-Glucanase also reduced intestinal viscosity and vent pasting. Both β-glucanase and flavomycin reduced percentage intestines, but the effects were not additive. In general, the effects of the enzyme preparations and flavomycin were independent, except for percentage intestines with β-glucanase.

(*Key words*: β-glucanase, xylanase, flavomycin, performance, thermostability of enzyme preparations)

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INTRODUCTION

Flavomycin (flavophospholipol) also known as moenomycin and bambermycin, is a fermentation product of *Streptomyces ghaenaensis* (Huber *et al.*, 1965). It is a P-containing glycolipid and is nonabsorbable (Wasiliewski *et al.*, 1965). Its growth promoting activity was repeatedly tested in 48 experiments, and its efficacy in broilers has been maintained 20 yr after it was first tested (Dost, 1985).

There is considerable evidence that the anti-nutritive activity of nonstarch polysaccharides in poultry is related to the gut microflora of the chicken, as addition of antibiotics to diets increases their nutritive value (Annison and Choct, 1991). On the other hand, the bioefficacy of enzymatic preparations containing β-glucanase and pentosanase activities in broiler diets containing barley or wheat is well established (Bedford and Morgan, 1996). However, there are very few studies in which the effects of the combination of antibiotic preparations and nonstarch polysaccharide degrading enzymes have been tested. Vukic Vranjes and Wenk (1995) studied the effects of avoparcin and an enzyme preparation containing β-glucanase and xylanase preparations in broiler diets containing barley. They found that the enzyme preparation improved performance, energy, fat, and nitrogen utilization and dry matter content of excreta. Avoparcin improved availability of energy and fat utilization. However, the inclusion of both supplements in the diet did not have a fully additive effect on performance, metabolizable energy, or fat and nitrogen utilization, suggesting that both supplements may have overlapping effects.

Stability of enzymes during the pelleting process has been a cause of concern. Yu and Tsen (1993) tested the stability of several proteases and carbohydrases in solution and in mixed feed and found that cellulase only

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TABLE 1. Composition of the experimental diets

Ingredients and contents	Barley		Wheat	
	Starter	Finisher	Starter	Finisher
	(%)			
Barley	53.334	58.489		
Wheat			50.000	50.000
Maize			7.841	11.010
Animal and vegetable fat	3.000	3.000	3.000	3.000
Full fat extruded soybeans	28.087	34.066	10.779	16.575
Soybean meal, 47.5% protein	11.531	1.070	24.230	16.055
DL-methionine	0.201	0.107	0.185	0.081
L-lysine HCl	0.013		0.106	0.036
L-threonine		0.030		
Calcium carbonate	1.175	1.310	1.195	1.302
Dicalcium phosphate	1.811	1.212	1.829	1.238
Salt	0.448	0.316	0.435	0.303
Minerals and vitamins[1]	0.400	0.400	0.400	0.400
Nicarbacin, mg/kg	125		125	
Salinomycin, mg/kg		60		60
Calculated nutrient content				
Metabolizable energy, kcal/kg	3,050	3,150	3,050	3,150
Crude protein	21.7	19.7	21.8	20.3
Lysine	1.20	1.05	1.20	1.05
Methionine + cystine	0.92	0.77	0.90	0.76
Calcium	1.00	0.90	1.00	1.00
Inorganic phosphorus	0.45	0.35	0.45	0.35

[1]One kilogram of feed contains: vitamin A, 12,000 IU; cholecalciferol, 5,000 IU; vitamin E, 30 mg; menadione, 3 mg; vitamin B_1, 2.2 mg; vitamin B_2, 8 mg; vitamin B_6, 5 mg; vitamin B_{12}, 11 mg; folic acid, 1.5 mg; biotin, 150 μg; calcium pantothenate, 25 mg; nicotinic acid, 65 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg; ethoxyquin, 150 mg.

lost 20% of its activity at 80 C for 5 min in solution, and very little in mixed feed, suggesting that the stability could be enhanced by mixing the enzymes in the feed. Viveros *et al.* (1994) tested the effects of autoclaving barley diets containing β-glucanase at 70 and 90 C for 10 min on performance of broiler chickens and found a significant improvement over unsupplemented diets, suggesting that β-glucanase resisted the autoclaving temperatures. Inborr and Bedford (1994) found that time and temperature above 75 C reduced β-glucanase activity contained in feed supplemented with a commercial preparation. They suggested that partial inactivation occurred during pelleting, although bird performance was only affected when pelleting temperatures exceeded 85 C. Finally, Almirall and Esteve-Garcia (1995) incubated a crude β-glucanase preparation from *Trichoderma brachiatum* in solution at different temperatures and found that at 70 C, activity was reduced to 65%, at 80 C activity was reduced to 20%, and at 100 C the solution was totally inactivated.

The objective of this study was to test the effects of an enzyme preparation containing nonstarch degrading enzymes and an antibiotic with growth promoting activities, and to determine whether these effects were independent. In addition, the stability of the enzyme preparations after pelleting was tested, because there is concern about the thermostability of enzyme preparations during pelleting (Chesson, 1987).

MATERIALS AND METHODS

Birds and Housing

Three thousand and six hundred sexed day-old broiler chicks of the Hybro strain were used. The chickens were distributed by sex into 48 pens, 8 m^2 each and housed at 75 chickens per pen. The pens were located in the two broiler houses on the farm. One house was used for the barley diets and the other for the wheat diets. The experiment was analyzed separately as two trials, one for the barley, and the other for the wheat diets. Each house is divided in two rooms of 12 pens each. The chicks were distributed by sex into each of the two rooms, so that one room contained only males and the other only females. In this way, the sex effect was confounded with the room effect. Therefore, sex was used as a blocking factor.

Experimental Diets

The feeding program consisted of two diets, starter and finisher. The starter diet provided 3,050 kcal ME/kg, and a minimum of 21% protein, 1.2% lysine, and 0.9% total sulfur amino acids, and was offered until 21 d of age in the wheat trial, and 22 d of age in the barley trial. The finisher diet provided 3,150 kcal ME/kg, and a minimum of 19% protein, 1.05% lysine, and 0.72% total sulfur amino acids, and was offered until the end of the trial. Nicarbacin was used as coccidiostat in the starter diet, and salinomycin in

TABLE 2. Analyzed compositions of the experimental diets

Diet	Moisture	Total β-glucans	Insoluble β-glucans	Pentosans	Crude fiber	Ether extract	Crude protein	NDF[1]	ADF[2]
	(%)								
Wheat diet									
Starter									
T-1	10.75[3]	0.36	0.26	6.92	3.62	6.57	22.17	10.64	4.39
T-2 F[4]	10.31	0.35	0.27	7.72	3.57	6.55	22.03	10.38	4.47
T-3 H[5]	10.21	0.35	0.26	7.52	3.29	6.75	21.89	9.47	4.33
T-4 F + H	10.35	0.36	0.28	7.39	3.57	6.78	21.63	9.65	4.36
Finisher									
T-1	10.69	0.35	0.30	7.33	3.52	7.53	20.19	11.57	4.17
T-2 F	10.39	0.38	0.27	7.23	3.39	7.42	20.39	12.51	4.29
T-3 H[6]	10.18	0.36	0.21	7.27	3.63	7.67	20.39	11.99	4.10
T-4 F + H	10.39	0.33	0.23	7.34	3.63	7.77	20.63	12.56	3.64
Barley diets									
Starter									
T-1	10.41	2.15	0.88	8.35	3.77	9.80	21.86	14.81	5.03
T-2 F	10.49	1.97	0.99	8.23	4.45	10.08	22.11	14.41	5.19
T-3 H[5]	11.09	1.99	0.82	8.68	4.42	9.80	22.73	15.13	5.23
T-4 F + H	11.51	1.94	0.73	7.55	4.19	9.64	22.25	14.04	5.33
Finisher									
T-1	11.97	2.18	0.81	7.93	4.07	10.62	20.08	13.38	4.66
T-2 F	11.42	2.21	1.02	8.47	4.44	10.49	20.21	13.79	5.11
T-3 H	10.84	2.21	1.02	9.00	4.50	10.14	20.14	14.48	5.42
T-4 F + H	10.89	2.17	0.92	8.58	4.43	10.84	20.26	14.33	5.26

[1]Neutral detergent fiber.

[2]Acid detergent fiber.

[3]Values are means of duplicate samples.

[4]Flavomycin at 4 mg/kg of feed.

[5]Hostazym® X at 1,500 endopentosanase units/kg of feed in wheat diets.

[6]Hostazym® C at 500 cellulase units/kg of feed in barley diets.

the finisher diet. Compositions of the basal experimental diets are shown in Table 1. Analyzed compositions of the experimental diets are shown in Table 2. Enzymes were mixed with wheat or barley, so that the premix containing them represented 2% of the total mix. All feed ingredients were ground through a 25 HP hammer mill to pass a 3-mm sieve, except minerals, vitamins, and additive premixes, which were added directly in the mixer. The mixer was a 500-kg capacity horizontal mixer, and the time of mixing was 5 min.

Pelleting was done in a 20 HP pelleting machine, using steam. The temperature in the conditioner was adjusted to 65 C and the capacity of the pelleting machine was adjusted to 25 A, about 80% of its available potency. Temperatures during the pelleting process were recorded in the conditioner, at the outlet of the die, and in the cooling tower. Temperatures recorded during pelleting are shown in Table 3.

Feeds were given in pelleted form. The starter pellets were 3 mm in diameter and were offered during the first 3 wk. The finisher pellets were 4 mm in diameter and were offered until the end of the experiment.

After the end of the trial, the chicks not selected for carcass measurements were fed a withdrawal diet for a minimum of 4 d. Chickens selected for carcass measurements were kept on the experimental diets for 5 d in the case of the wheat experiment and for 4 d in the case of the barley experiment. Feed was administered for *ad libitum* consumption throughout the experiment.

Experimental Design and Statistical Analyses

Each experiment was designed and analyzed as a factorial arrangement of treatments with two levels of flavomycin (0 and 4 mg/kg) and two levels of enzyme preparations containing mainly xylanase [1,500 endopentosanase units (EPU)/kg] or β-glucanase[2] [500 cellulase units (CU)/kg] for the wheat and barley experiments, respectively. Therefore, the arrangement of treatments was T-1, negative control; T-2, control + flavomycin; T-3, control + enzyme preparation; T-4, control + flavomycin + enzyme preparation. There were three replicate pens of 75 chicks per sex and within each sex. As mentioned, sex was used as a blocking factor. Location within the house was also considered as a blocking factor. A set of linear orthogonal contrasts was used to test the effects of enzyme (T-1 and T-2 vs T-3 and T-4; T-5 and T-6 vs T-7 and T-8); flavomycin (T-1 and T-3 vs T-2 and T-4; T-5 and T-7 vs T-6 and T-8); and the interaction (T-1 and T-4 vs T-2 and T-3; T-5 and T-8 vs T-6 and T-7). In addition, Duncan's multiple

[2]Xylanase was supplemented as Hostazym® X microgranulate containing 3,000 EPU/kg, and β-glucanase as Hostazym® C microgranulate containing 1,000 CU/g from Hoechst-Roussel Veterinär, Wiesbaden, Germany.

TABLE 3. Temperature of the feed during pelleting[1]

Treatments	Conditioner	Outlet of die	Cooler
	(C)		
Wheat diets			
Starter			
T-1	63 to 65	68 to 78	39 to 58
T-2 F[2]	64 to 65	72 to 79	31 to 58
T-3 H[3]	63 to 66	73 to 80	34 to 56
T-4 F + H	63 to 65	74 to 80	33 to 56
Finisher			
T-1	64 to 65	57 to 72	25 to 47
T-2 F	63 to 65	61 to 73	28 to 58
T-3 H	63 to 65	69 to 75	35 to 58
T-4 F + H	63 to 65	68 to 74	32 to 60
Barley diets			
Starter			
T-1	63 to 66	69 to 75	36 to 58
T-2 F	63 to 66	71 to 76	33 to 59
T-3 H[4]	63 to 66	69 to 74	35 to 51
T-4 F + H	64 to 67	57 to 71	27 to 48
Finisher			
T-1	63 to 67	58 to 69	25 to 43
T-2 F	62 to 66	57 to 69	30 to 52
T-3 H	61 to 66	60 to 72	27 to 52
T-4 F + H	63 to 66	65 to 71	33 to 54

[1]Range of temperatures recorded during the pelleting process.

[2]Flavomycin at 4 mg/kg of feed.

[3]Hostazym® X at 1,500 endopentosanase units/kg of feed in wheat diets.

[4]Hostazym® C at 500 cellulase units/kg of feed in barley diets.

range test was performed to separate treatment means as if treatments were completely randomized, instead of a factorial arrangement, in cases in which significant interactions between flavomycin and the enzyme preparations were detected. For carcass determinations, data expressed as percentages were transformed to the arc sine of the square root of the percentage divided by 100, to make the variance more homogeneous.

Controls

Performance. Animals were weighed as a group by sex on arrival. Animals and feed were weighed by pen on Day 21 for the wheat and on Day 22 for the barley experiment, and at the end of the experiment at 42 and 43 d of age. Average final weight, daily gain, feed consumption, and feed to gain was determined for each period and for the overall experiment. Average daily gain was calculated as the difference between the average of live bird at the end of the period and the average of live bird beginning of the period, divided by the number of days. Dead animals were not taken into account, and their weights were subtracted from the initial weight of the pen, according to the mean weight, or, in case the animal was smaller than the initial weight due to disease, its weight at the time of death was subtracted from the initial weight of the pen. Average daily feed intake was calculated as the total feed consumed within the period divided by the number of days and animals, subtracting the amount of estimated feed consumed by the dead animals. This was estimated according to the weight gained by the dead animal within the period, the number of days, and the estimated feed conversion within the period the dead animal was present. Average feed conversion rate was calculated dividing the total feed consumed within the period by the weight gained by the live animals within the period. Day, location within the house, and replicate within a day were used as blocking factors.

Vent Pasting. Vent pasting was evaluated by visual observations of the chickens individually at 10 d of age, by a single monitor, to ensure homogeneity of criteria. Results were expressed as a percentage of animals showing adhered droppings to the perineal region.

Carcass Measurements. At the end of the experiment, four chicks per pen were wing-banded and continued consuming the experimental diets until 47 d of age, when they were again weighed individually. These chickens were slaughtered separately. The carcasses were chilled, and carcass yield, viscera (including gizzard, proventriculus, liver, spleen, and intestine), intestines, and abdominal fat pad as a proportion of live body weight were measured the following day.

Intestinal Viscosity. Intestinal viscosity was determined in a separate group of chickens with the same diets as for the performance study. One hundred and ninety-two day-old male broiler chicks were randomly distributed into 16 pens, at 12 chicks per pen, in a flat deck battery house. There were two blocks of pens within the room. The chickens were fed the experimental diets for 14 d, starting when the chicks were 1 wk old. At 3 wk of age, intestinal viscosity was determined on 3 consecutive d. Chickens were killed by intravenous injection of 0.5 mL of 5% thiobarbital. Two (Days 1 and 2) or three (Day 3) samples per pen were obtained. Each sample consisted of the intestinal content of two chickens from one pen, between Meckel's diverticulum and the ileocecal junction. The samples were centrifuged at $10,000 \times g$ for 5 min, and the viscosity of the supernatant determined at room temperature and a shearing rate between 11.25 and 450 s^{-1} in a Brookfield digital viscometer, Model DV-II.[3] Data from viscosity measurements were transformed to log of viscosity to make the variance more homogeneous.

Feed Analyses. Feeds were analyzed for enzyme activities and flavomycin contents. Flavomycin was analyzed according to the method of Hoechst Roussel Vet for mycelium, premixes, and feedstuffs containing more than 0.5 mg of flavomycin/kg. The method consists of an extraction with methanol, purification by ion exchange chromatography in Dowex columns, and quantitative determination in agar diffusion gel as a parallel diffusion test for three concentrations of standard solutions and sample extracts. β-Glucanase activity was analyzed according to the method of Hoechst Roussel Vet for cellulase

[3]Brookfield Engineering Laboratories, Inc., Stoughton, MA 02072.

TABLE 4. Analytical composition of the feed: enzyme activities and flavomycin

Diet	Form	Product	Units[1]	Expected	Found	Recovery
						(%)
Wheat diets						
Starter						
T-2	Mash	Flavomycin	mg/kg	4	4	
	Pellet		mg/kg	4	3.4	
T-3	Mash	Hostazym® X micro Granulate	EPU/kg	1,500	2,487	
	Pellet	Hostazym® X micro Granulate	EPU/kg	1,500	2,086	83.9
T-4	Mash	Flavomycin	mg/kg	4	3.8	
		Hostazym® X micro Granulate	EPU/kg	1,500	1,998	
	Pellet	Flavomycin	mg/kg	4	3.6	
		Hostazym® X micro Granulate	EPU/kg	1,500	1,827	91.4
Finisher						
T-2	Mash	Flavomycin	mg/kg	4	3.8	
	Pellet		mg/kg	4	3.7	
T-3	Mash	Hostazym® X micro Granulate	EPU/kg	1,500	2,101	
	Pellet	Hostazym® X micro Granulate	EPU/kg	1,500	2,244	106.8
T-4	Mash	Flavomycin	mg/kg	4	3.7	
		Hostazym® X micro Granulate	EPU/kg	1,500	2,030	
	Pellet	Flavomycin	mg/kg	4	3.7	
		Hostazym® X micro Granulate	EPU/kg	1,500	1,603	79.0
Barley diet						
Starter						
T-2	Mash	Flavomycin	mg/kg	4	3.5	
	Pellet		mg/kg	4	3.6	
T-3	Mash	Hostazym® C micro Granulate	CU/kg	500	1,213	
	Pellet	Hostazym® C micro Granulate	CU/kg	500	1,287	106.1
T-4	Mash	Flavomycin	mg/kg	4	3.0	
		Hostazym® C micro Granulate	CU/kg	500	1,007	
	Pellet	Flavomycin	mg/kg	4	3.3	
		Hostazym® C micro Granulate	CU/kg	500	1,047	103.8
Finisher						
T-2	Mash	Flavomycin	mg/kg	4	4	
	Pellet		mg/kg	4	3.7	
T-3	Mash	Hostazym® C micro Granulate	CU/kg	500	484	
	Pellet	Hostazym® C micro Granulate	CU/kg	500	411	84.8
T-4	Mash	Flavomycin	mg/kg	4	3.8	
		Hostazym® C micro Granulate	CU/kg	500	580	
	Pellet	Flavomycin	mg/kg	4	3.2	
		Hostazym® C micro Granulate	CU/kg	500	507	87.4

[1]EPU = endopentosanase units. CU = cellulase units.

in feed. The sample is spiked with different amounts of cellulase reference standard, and the cellulase is extracted with acetate buffer. The activity of the enzyme is determined by incubation of the extract with Azurine cross-linked cellulose as substrate and photometric measurement of the released color complex. The conditions of the reaction are at pH 4.5 and 50 C for 2.5 h. The cellulase activity is calculated following the Standard Addition Method, which consists in calculating the regression line of absorbance vs cellulase addition, and determination of the abscissa when absorbance = 0.

Pentosanase activity was determined by a similar method, using an endopentosanase reference standard, and incubation with Azurine cross-linked xylan as substrate. The conditions of the reaction are at pH 4.7 and 50 C for 2.5 h. The pentosanase activity is also calculated following the Standard Addition Method.

Xylanase activity was determined in the T-1, T-3, and T-4 diets of the wheat experiment in the mash before pelleting and in the pellets. β-Glucanase activity was determined in the T-5, T-7, and T-8 of the barley feed in the mash before pelleting and in the pellets. Feed were also analyzed for crude protein, ether extract, crude fiber, acid detergent fiber, and neutral detergent fiber according to the AOAC procedures (AOAC, 1990). β-Glucans and pentosans were determined according to the procedures of McCleary and Glennie-Holmes (1985) and Hashimoto *et al.* (1987), respectively.

RESULTS

Stability of Enzymes in Pelleted Feed

Temperatures during pelleting are shown in Table 3 and the stability of enzymes in Table 4. Enzyme recoveries represent the percentage of the activity present in the final mash feed, and therefore indicate the actual resistance of the enzymes to the pelleting conditions. Flavomycin content was close to the expected results. Enzyme activities were, in some cases, higher than expected, but resistance to pelleting conditions was between 80 and 100% of the measured quantities in the mash feed, indicating good thermostability under the conditions of pelleting employed.

TABLE 5. Body weight, and feed to gain ratio (FE) of chickens fed wheat diets containing different concentrations of flavomycin (F) and endopentosanase (EP)

Dietary treatment		Age					
		21 d		42 d			
F	EP	BW	FE	BW	FE	Livability	Vent pasting
(mg/kg)	(units/kg)	(kg)		(kg)		(%)	
0	0	569	1.680	1,951	1.973	96.5	6.6
4	0	597	1.626	2,000	1.951	96.7	6.9
0	1,500	571	1.634	1,955	1.918	97.3	3.7
4	1,500	595	1.569	2,000	1.884	96.7	3.1
SEM		5.1	0.011	17.3	0.009	0.69	1.22
Source of variation		Probabilities					
F		0.0001	0.0001	0.01	0.01	0.77	0.87
EP		0.96	0.001	0.93	0.0001	0.62	0.01
F by EP		0.71	0.61	0.90	0.51	0.62	0.71
Main effect means							
F							
0		570[B]	1.657[A]	1,953[B]	1.946[A]	96.4	5.1
4		596[A]	1.598[B]	2,000[A]	1.918[B]	96.7	5.0
EP							
0		583	1.653[A]	1,975	1.962[A]	96.6	6.7[A]
1,500		583	1.602[B]	1,977	1.901[B]	96.5	3.4[B]

[A,B]Means with no common superscript differ significantly ($P < 0.01$).

[1]Means of six pens per treatment, 75 chickens at the start.

Wheat Diets

Performance is shown in Table 5. Between 0 and 21 d, flavomycin had a significant effect on weight gain ($P < 0.0001$) and on feed conversion. The xylanase preparation improved feed conversion ($P < 0.001$). Incidence of vent pasting was significantly reduced by the xylanase preparation ($P < 0.01$). Between 21 and 42 d, chicks receiving flavomycin achieved a greater final weight ($P < 0.01$) and the effect on weight gain during this period approached significance ($P = 0.10$). Overall, flavomycin significantly improved daily gain and feed conversion ($P < 0.01$). The xylanase preparation significantly improved feed conversion ($P < 0.0001$).

Viscosity measurements are shown in Table 6. Both the xylanase preparation and flavomycin reduced intestinal viscosity ($P < 0.05$ and $P < 0.06$), respectively. Carcass measurements are shown in Table 7. The xylanase preparation reduced the percentage viscerae ($P < 0.05$). This result was mostly due to a reduction in percentage intestines, although the difference was not significant ($P < 0.12$). Abdominal fat tended to decrease ($P < 0.07$) with the inclusion of flavomycin. Effects of flavomycin and xylanase were always independent, and no significant interactions were detected, except for carcass yield, for which a significant interaction between flavomycin and endopentosanase was detected.

Barley

Performance with the barley diet is shown in Table 8. In the period 0 to 21 d, flavomycin improved daily gain ($P < 0.001$) and feed conversion ($P < 0.0001$). The β-glucanase preparation also improved weight gain and feed conversion ($P < 0.0001$). Incidence of vent pasting was greatly reduced by the β-glucanase preparation ($P < 0.0001$). Between 22 and 42 d, the β-glucanase preparation and flavomycin improved feed conversion ($P < 0.01$). Overall, feed conversion was greatly improved by β-glucanase and flavomycin ($P < 0.0001$).

Viscosity measurements are shown in Table 9. The β-glucanase preparation reduced intestinal viscosity ($P < 0.05$). Carcass measurements are shown in Table 10. There was a significant interaction between β-glucanase and flavomycin, in the sense that both reduced percentage viscerae and intestines, but the effect of flavomycin was not additive to that of β-glucanase, and occurred only in its absence.

DISCUSSION

The results of enzyme stability in feed indicate that enzyme activities were, in some cases, higher than expected, but the activities measured in pellets were between 80 and 100% of the measured quantities in the mash feed, indicating good thermostability under the conditions of pelleting employed. Considering that the temperatures reached at the outlet of die were in some cases around 80 C (Table 3) and that there was no apparent difference due to the different temperatures achieved (slightly higher in the starter diets, possibly due to the lower fat content), the results indicate that the enzymes maintained over 80% of their activity at

TABLE 6. Intestinal viscosity of chickens fed wheat diets containing different concentrations of flavomycin (F) and endopentosanase (EP)

Treatment			
F	EP	Viscosity	Log of viscosity
(mg/kg)	(units/kg)	(cps)	
0	0	3.6[1]	0.95
4	0	2.1	0.65
0	1,500	1.9	0.64
4	1,500	1.6	0.45
SEM			0.137
Source of variation			Probabilities
F			0.05
EP			0.06
F × EP			0.66
Main effect means			
F			
0		2.7[a]	0.79
4		1.9[b]	0.60
EP			
0		2.9[a]	0.80
1,500		1.7[b]	0.55

[a,b]Means with no common superscript differ significantly ($P < 0.06$).

[1]Values are means of 12 determinations per treatment.

temperatures as high as 80 C during pelleting. It is possible that the resistance of the enzyme preparations to temperature is greater in feed than in solution, as suggested by Yu and Tsen (1993).

Flavomycin significantly improved daily gain during the first 3-wk period, in both the wheat and the barley diets. Flavomycin also improved feed conversion during this period; however, in the second period, its effects were significant only in the barley diets. Flavomycin has been shown to enhance the digestibility of protein and some amino acids, such as methionine (Valerani, 1982), and this could be related to the improved performance caused by flavomycin. The results also confirm the reported effects of flavomycin on performance (Dost, 1985). The effect of flavomycin on feed efficiency was more pronounced in diets containing barley than in the diets containing wheat. Antoniou and Marquardt (1982) noted that penicillin supplementation considerably improved the performance of broiler diets containing high levels of rye but had little effect in diets containing high levels of wheat. This result and those of the present study suggest that wheat diets are less susceptible that barley diets to the effects of antibiotic with growth promoter activities, and that the effects of these types of products may be related to their effects on the intestinal microflora (Annison and Choct, 1991).

The xylanase preparation reduced feed intake in the wheat diets throughout the experiment, and improved feed conversion during all periods. This result suggests that the endoxylanase activity present in the enzyme preparation improved the utilization of nutrients and improved feed efficiency. Wheat contains arabinoxylans, which are known to have an anti-nutritive effect on broilers and to decrease energy and nitrogen availability (Choct and Annison, 1990, 1992a). Endoxylanases have been shown to improve the performance of broilers fed wheat diets (Annison, 1992; Flores *et al.*, 1994; Veldman and Vahl, 1994; Choct *et al.*, 1995) by degrading the viscous nonstarch polysaccharides present in wheat and to improve energy, starch, and pentosan digestibility (Annison, 1992).

The wheat diets contained a considerable level of

TABLE 7. Carcass measurements of chickens fed wheat diets containing different concentrations of flavomycin (F) and endopentosanase (EP)

Treatment						
F	EP	Live body weight	Carcass yield	Percentage viscera	Percentage intestines	Percentage abdominal fat
(mg/kg)	(units/kg)	(g)	(%)			
0	0	2,447[1]	77.1[ab]	9.09	5.03	2.77
4	0	2,486	76.6[a]	9.13	5.20	2.60
0	1,500	2,507	76.8[a]	8.80	4.95	2.72
4	1,500	2,464	77.5[b]	8.76	4.94	2.58
SEM		23.9	0.15	0.080	0.081	0.057
Source of variation		Probabilities				
F		0.95	0.57	0.96	0.37	0.07
EP		0.48	0.18	0.05	0.12	0.70
EP × F		0.19	0.03	0.62	0.37	0.83
Main effect means						
F						
0		2,477	76.9	8.95	4.99	2.75[a]
4		2,475	77.1	8.95	5.07	2.59[b]
EP						
0		2,481	76.9	9.11[a]	5.11	2.69
1,500		2,485	77.1	8.78[b]	4.95	2.65

[a,b]Means with no common superscript differ significantly ($P < 0.07$).

[1]Values are means of 12 determinations per treatment.

TABLE 8. Body weight, feed to gain ratio (FE) of chickens fed barley diets containing different concentrations of flavomycin (F) and cellulase (C)

Dietary treatment		Age					
		21 d		42 d			
F	C	BW	FE	BW	FE	Livability	Vent pasting
(mg/kg)	(units/kg)					(%)	
0	0	578[1]	1.741[A]	1,881	2.021	97.5	58.7
4	0	590	1.721[B]	1,885	1.986	97.3	56.1
0	500	595	1.692[C]	1,856	1.947	97.3	11.2
4	500	618	1.641[D]	1,949	1.907	98.0	8.4
SEM		4.5	0.005	26.1	0.005	0.76	2.06
Source of variation		Probabilities					
F		0.001	0.0001	0.10	0.0001	0.74	0.87
C		0.0001	0.0001	0.50	0.0001	0.74	0.01
F × C		0.21	0.01	0.14	0.72	0.58	0.71
Main effect means							
F							
0		587[B]	1.717[A]	1,869	1.984[A]	97.4	34.9
4		604[A]	1.681[B]	1,917	1.947[B]	97.6	32.3
C							
0		584[A]	1.731[A]	1,883	2.004[A]	97.4	57.4[A]
500		606[B]	1.667[B]	1,903	1.927[B]	97.6	9.8[B]

[A-D]Means with no common superscript differ significantly ($P < 0.01$).

[1]Means of six pens per treatment, 75 chickens at the start.

pentosans (about 7%), indicating that the wheat used was rich in pentosans. In fact, the barley diets contained even slightly higher levels of pentosans. In contrast, the levels of β-glucans in the wheat diets were rather low, which suggests that the improvements caused by the xylanase preparation were due to a reduction in pentosan concentration in the digestive tract, as indicated by the reduction of viscosity of the intestinal content in the wheat diets. In wheat diets, van der Klis *et al.* (1995) showed an effect of viscosity caused by xylans on dry matter and mineral absorption in broilers. Other researchers have reported that a decrease in viscosity was observed in parallel to an improvement in feed conversion ratio (Veldman and Vahl, 1994) and in energy and starch digestibility (Choct *et al.*, 1995).

The β-glucanase preparation reduced feed consumption only in the second period in chicks fed the barley diets, yet feed conversion was markedly improved by the β-glucanase preparation. The beneficial effect of β-glucanase addition to barley diets for broilers is well documented (for example, Brufau *et al.*, 1991; Francesch *et al.*, 1994; Almirall and Esteve-Garcia, 1994; Almirall *et al.*, 1995). The concentration of β-glucan in the barley diets can be considered normal, but it is important to note that the presence of pentosans was also high, suggesting that the effects of the enzyme preparation can be caused by a decrease in β-glucan as well as pentosan concentration along the digestive tract. However, the greater viscosity observed in the barley diets indicates that β-glucans are responsible for the major part of the viscosity observed in the intestine, and that the reduction in viscosity is brought about by β-glucanase, in agreement with previous observations (Veldman and Vahl, 1994; Almirall *et al.*, 1995).

Incidence of vent pasting was more pronounced in barley than in wheat diets, and the reduction caused by enzyme addition was more dramatic for the β-glucanase than for the xylanase preparation. This result strongly suggests that this phenomenon is closely related to viscosity. The effects of β-glucanase on intestinal vis-

TABLE 9. Intestinal viscosity of chickens fed barley diets containing different concentrations of flavomycin (F) and cellulase (C)

Treatment			
F	C	Viscosity	Log of viscosity
(mg/kg)	(units/kg)	(cps)	
0	0	8.2	1.94
4	0	19.6	2.36
0	500	2.3	0.74
4	500	2.9	0.85
SEM			0.18
Source of variation			
F			0.13
C			0.0001
F × C			0.37
Main effect means			
F			
0		5.3	1.34
4		11.3	1.61
C			
0		13.9[A]	2.15[A]
500		2.5[B]	1.45[B]

[A,B]Means with no common superscript differ significantly ($P < 0.0001$).

[1]Values are means of 12 determinations per treatment.

TABLE 10. Carcass measurements of chickens fed barley diets containing different concentrations of flavomycin (F) and cellulase (C)

Treatment		Live body weight	Carcass[1] yield	Percentage viscera	Percentage intestines	Percentage abdominal fat
F	EP					
(mg/kg)	(units/kg)	(g)	(%)			
0	0	2,235	78.5	10.6[a]	5.45[a]	2.67
4	0	2,264	76.7	9.2[b]	4.65[b]	2.51
0	1,500	2,268	76.8	8.7[c]	4.30[c]	2.80
4	1,500	2,340	76.7	8.7[c]	4.30[c]	2.82
SEM		59.2	0.72	0.12	0.097	0.123
Source of variation						
F		0.46	0.27	0.05	0.05	0.78
C		0.43	0.33	0.01	0.01	0.28
F × C		0.74	0.32	0.05	0.05	0.58
Main effect means						
F						
0		2,251	77.7	9.7[a]	4.87[a]	2.73
4		2,302	76.7	8.9[b]	4.47[b]	2.67
EP						
0		2,249	77.6	9.9[a]	5.05[a]	2.59
1,500		2,304	76.7	8.7[b]	4.30[b]	2.81

[a-c]Means with no common superscript differ (Duncan's multiple range test at $P = 0.05$).
[1]Values are means of 12 determinations per treatment.

cosity are also well known (Almirall *et al.*, 1995) as well as its relationship to performance and nutrient digestibility (Almirall *et al.*, 1995).

Flavomycin showed an additive effect on feed efficiency to that of the enzyme preparations. However, it is interesting to note that in the wheat diets, flavomycin tended to increase feed intake and xylanase tended to reduce it, suggesting that the effects of both products on feed intake may not be related to their effects on viscosity. Research on the combined effects of antibiotics and enzymes is rather scarce. Antoniou and Marquardt (1982) found that penicillin supplementation improved performance and lipid digestibility of rye-based diets, but that the effects on wheat-based diets were much smaller, and suggested a possible role of the intestinal microflora in reducing lipid digestibility. Choct and Annison (1992b) did not find any improvement by supplementing diets containing wheat pentosans with procaine penicillin. The same authors (Annison and Choct, 1991) suggested that suppression of intestinal microflora that compete with the host may be responsible for the positive effects of antibiotics found in some cases. Finally, Vukic Vranjes and Wenk (1995) reported an interaction between avoparcin and an enzyme complex for feed efficiency in the first experimental period (7 to 21 d), and suggested that effects on the microflora caused by antibiotic supplementation could be responsible for the improvements in fat availability. They also found a significant interaction between the two factors for fiber degradability. The results of the present experiment suggest that, for the most part, the effects of flavomycin and enzymes are independent of each other.

Carcass yield was not significantly affected by either flavomycin or the enzyme preparations, but percentage viscera was reduced by the enzymes in both the wheat and the barley diets. This reduction was largely caused by a reduction in the percentage of intestines. Flavomycin also affected the barley diets, but its effect was apparent only when β-glucanase was absent. In fact, this was the only case when an interaction between flavomycin and β-glucanase was significant, and suggests that the reduction in percentage intestines may be brought about by the same mechanism, possibly a reduction in intestinal microflora, either by direct effect of the antibiotic, or by a reduction in nonstarch polysaccharides.

Supplementation with both enzyme preparations improves the performance of broiler chickens fed wheat or barley diets, respectively, and in the presence of flavomycin. Flavomycin tended to increase weight gain and to improve feed conversion. Xylanase and β-glucanase tended to improve feed conversion. In general, the effects of flavomycin and enzyme are independent, and no significant interactions were observed in performance. Enzymes show important effects on intestinal viscosity, but these effects do not seem to be related to feed intake. Enzymes also tend to reduce the percentage of viscera.

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